Peer Review
Experimental Toxicology Division
NHEERL

Influence of Diabetes and Body Fat on the Pharmacokinetics of Dioxin in Rodents

SUSCEPTIBILITY, ALLERGY & ASTHMA

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INTRODUCTION

- Type II diabetes is increasing in the US population.
- Type II diabetes is associated with obesity.
- In type II diabetics, there is evidence of altered expression of xenobiotic metabolizing enzymes.
- Both body fat mass and expression of xenobiotic metabolizing enzymes are important factors in the absorption, distribution, metabolism and elimination (ADME) of environmental chemicals.
- Because of alterations in body fat mass and xenobiotic metabolizing enzymes, the ADME of environmental chemicals may be altered in type II diabetics. This may result in altered risks to environmental chemicals in type II diabetics.

GOAL

To examine effects of type II diabetes on the pharmacokinetics of environmental chemicals.

- TCDD, initial test chemical:
  - Epidemiological studies show a relationship between TCDD exposure and diabetes.
  - Epidemiological studies show that TCDD elimination decreases as body fat increases.
  - Type II diabetes associated with increased body fat mass.
- Characterize expression of xenobiotic metabolizing enzymes in models of type II diabetes:
  - Examine expression of both phase I and II xenobiotic metabolizing enzymes.
  - Examine expression of these enzymes in hepatic and extrahepatic tissues.

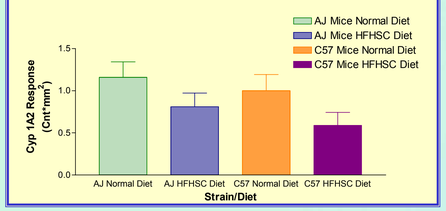
MATERIALS and METHODS

- 4 WEEK OLD MALE C57BL/6J AND AJ/6 MICE WERE PLACED ON 2 DIFFERENT DIETS FOR 13 WEEKS PRIOR TO EXPOSURE TO TCDD BY ORAL GAVAGE.
  - NORMAL DIET (Purina Rodent Chow).
  - DIABETIC DIET (36% fat, 35% simple carbohydrates, 20.5% protein; HFHSC; #1850 BioServ)
  - 13 wks after being placed on diet, mice were exposed to 0, 0.1, or 5.0 µg [^{3}H]TCDD/kg and terminated 1, 3, 10, 20, 30, 40, or 60 days later.
  - TCDD tissue concentrations were determined by combustion and liquid scintillation counting.
  - Western Blot: CYP1A2 expression analyzed densitometrically; CYP1A2 specific antibody K-15 goat anti mouse (Santa Cruz Biotechnology).
- *In Vitro* Assay: Comparison of metabolism of prototype CYP1A2 substrates in rat and human SUPERSOMES (GenTest Corp.) and ability of TCDD to inhibit these reactions. Substrates: MROD (methoxyresorufin O-deethylase), ACOH (acetanilide 4-hydroxylase), and caffeine.

Influence of Obesity and Diabetes on the Half-Life of TCDD

Strain	C57BL/6J Mice				AJ/6 Mice			
Dose	0.1 µg TCDD/kg		5.0 µg TCDD/kg		0.1 µg TCDD/kg		5.0 µg TCDD/kg	
Diet	Normal	HFHSC	Normal	HFHSC	Normal	HFHSC	Normal	HFHSC
$t_{1/2}$ Blood (days)	14.1	185.5	15.8	28.4	25.6	23.6	17.1	19.2
$t_{1/2}$ Liver (days)	8.4	64.1	9.8	17.2	16.6	21.1	11.4	13.2
$t_{1/2}$ Fat (days)	14.7	73.2	12.5	28.1	26.2	30.7	20.3	21.9

Influence of Diet on CYP1A2 Expression



% Body Fat

Mice	Diet	% Body Fat (mean ± std)
C57BL/6J	Normal	10.1 ± 2.6
	HFHSC	34.3 ± 3.9
AJ/6	Normal	11.9 ± 2.5
	HFHSC	22.8 ± 6.7

Influence of CYP1A2 on the Pharmacokinetics of TCDD

Comparison of Metabolism in Rat and Human CYP1A2 Supersomes and Their Inhibition by TCDD

Substrate	Vmax			KM		
	Human	Rat	Unit	Human	Rat	Unit
MROD	1.8	2.8	pmol/min/pg P450	2.5	1.9	µM
ACOH	97.7	156	pmol/min/pg P450	15	50.2	mM
Caffeine	0.5	0.09	pmol/min/pg P450	11.7	10.6	mM

Tissue Distribution in TCDD-Dosed Mice

Tissue	Knockout Mice % dose [ng TCDD/g]	C57BL/6N Mice % dose [ng TCDD/g]	129/Sv Mice % dose [ng TCDD/g]
Liver	2.88 ± 0.55 [12.66 ± 2.44]	29.15 ± 2.04 [117.79 ± 13.00]	31.56 ± 2.83 [166.75 ± 6.66]
Adipose Tissue	42.15 ± 8.98 [74.11 ± 14.81]	11.79 ± 1.26 [33.29 ±2.87]	16.91 ±1.45 [46.05 ± 3.63]

Diliberto, J.J. *et al.*, (1999) **Toxicol. Appl. Pharmacol.** *159*, 52-64.

Overall Metabolism of TCDD

	C57BL/6N Mice % Dose	CYP1A2 Knockout Mice % Dose
0-96h Urine	3.36	2.42
0-96h Feces (extractable)	3.16	2.24
0-96h Feces (non-extractable)	4.62	1.26
Total Metabolism	11.14	5.92

Hakk, H. and Diliberto, J. J. (2002) *Organohalogen Compounds* **55**, 461-464.

CONCLUSIONS

- Diabetic/TCDD study
  - Obesity and type II diabetes increased the half-life of TCDD.
  - The effect of obesity and type II diabetes on the TCDD $t_{½}$ was dose dependent and more pronounced at the lower exposure.
  - A HFHSC diet decreased level of basal expression of hepatic CYP1A2 in both strains of mice.
  - CYP1A2 expression decreased more in C57BL/6J mice compared to AJ/6 mice.
- *In Vitro* metabolism of prototype substrates
  - TCDD inhibits CYP1A2 mediated metabolism of methoxyresorufin, acetanilide and caffeine.
  - Similar TCDD inhibition in rat and human CYP1A2 supersomes.
- CYP1A2 knockout mice
  - Hepatic sequestration in wild types but not knockouts.
  - Different disposition for TCDD and dioxin-like compounds between wild type and knockout mice.
  - An increased TCDD metabolism in wild type compared to CYP1A2 knockout mice.

IMPLICATIONS

- Data suggest that the pharmacokinetics of environmental chemicals are different in type II diabetics.
- Data suggest that the association between dioxin exposure and increased incidence of diabetes may be due in part to the influence of this disease state on the elimination of TCDD.

FUTURE DIRECTIONS

- Further characterizing expression and activity of phase I and II xenobiotic metabolizing enzymes in mouse and rat models of obesity and diabetes.
- Test the effects of diabetes on the pharmacokinetics of TCDD in rats to examine species concordance in this response.
- Develop a physiologically-based pharmacokinetic model for diabetes and obesity.

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